Available online at www.sciencedirect.com

ScienceDirect

Journal of Photochemistry and Photobiology A: Chemistry 196 (2008) 254–261

Journal of Photochemistry and Photobiology A:Chemistry

www.elsevier.com/locate/jphotochem

Bacteriorhodopsin—Perspective biomaterial for molecular nanophotonics

G.E. Adamov [*], A.G. Devyatkov, L.N. Gnatyuk, I.S. Goldobin, E.P. Grebennikov

OJSC "CSRIT Technomash", Ivan Franko str 4, 121108 Moscow, Russian Federation, Russia

Abstract

Results of investigation of photochromic protein bacteriorhodopsin (BR) extracted from cellular membranes of *Halobacterium salinarum* are represented. Basic processes and original approaches of neural networks realization based on bacteriorhodopsin in composition of multilayer structures. Requirements of BR-containing functional films are determined and results of investigation of layers modifications are represented for the purpose of stabilization of extremely high photochromic sensitivity of BR-containing media. The modification of BR-films using bifunctional crosslinking reagents to increase of technological parameters of BR is applied. The influence of 1,4-diaminobenzene, glutaric dialdehyde and lysine on photochromic properties of the bacteriorhodopsin films is investigated. Shown that addition of bifunctional crosslinking reagents increase technological parameters of materials based on bacteriorhodopsin.

Keywords: Molecular nanophotonics; Bacteriorhodopsin; Neural networks; Multilayer structures

1. Introduction

Two principal problems inevitably limit the efforts to create information-logical equipment at molecular level.

The first problem is that nanodevices formation by direct effect on single molecule or atom is technologically lacking in prospects. The essential time of single molecule operating results to the enormous time losses for the whole device formation and excessive inputs.

The second problem—The necessity of application of "macro–micro" and "micro–macro" interfaces for the primary information input and the following results output. The advantages of low-sized molecular elements disable due to the access baffling complexity.

Obviously, the only effective method of molecular system connection is the optical effect. In the strict sense this effect is directed not at the single molecule but at some fragment of substance, as the optical tools provide interaction with the object at resolution not better than one-half of the wave length, that is 200–300 nm. The solution of these problems can be found apparently at the hierarchical structural–functional self-organization of molecular systems, using molecular ensembles as a base for molecular information-logical devices. The biological material considered below—protein bacteriorhodopsin possesses the unique technological abilities, and its optical properties allow using optical in/out devices and to create molecular devices based on self-organization principles.

2. Bacteriorhodopsin and its properties used for the information processing

Bacteriorhodopsin—light-sensitive protein, similar to optic rhodopsin of human eye. BR is obtained of halobacteria-containing BR in cellular membranes (so-called, "purple membranes"). During the separation from the bacteria cells purple membranes save the structure entirely [1,2]. The typical size of purple membranes is 500–1000 nm. It is the unique biocrystalline structure capable to save its permanent properties during the number of years consisting of dry and polymer films with the thickness from 5 nm (monolayer) up to a few tens of μm.

The fundamental BR property is the photochemical cycle availability: after the light quantum absorption, BR molecule passes through the sequence of states and spontaneously returns to the primary form [3]. For some BR types the existence of branched photocycles is characteristic (Fig. 1) [4]. At that, in compliance with the cycling of BR molecule state, the

---

[*] Corresponding author. Tel.: +7 495 144 68 35; fax: +7 495 144 75 95.
E-mail address: ntc_technology@socket.ru (G.E. Adamov).

1010-6030/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jphotochem.2007.07.027

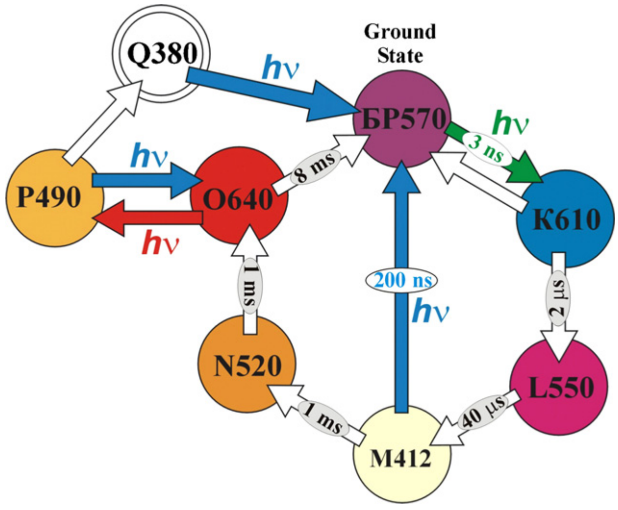

Fig. 1. Bacteriorhodopsin photocycle.

light-induced cycling of optical characteristics (refraction and absorption indices) occurs [5]. Each of the interstitial states is identified as the intermediate according to its absorption spectrum.

The main BR function in purple membranes—light-dependent proton transfer (H^+) over the purple membrane resulting to electrochemical hydrogen potential formation on halobacteria membrane, the energy of the potential is utilized by cell. Ejection of H^+ occurs outside the cell membrane, and the capture of H^+ comes on inside of the cell (from cytoplasm). It is supposed, that it takes place during the formation and the disappearance of intermediate M412 (Fig. 1) [3,6].

BR spectral sensitivity lies in optical band (Fig. 2) [4]. Absorption maximum in primary state BR570 corresponds to wavelength 570 nm. The main intermediate state M412 has the absorption maximum at wavelength 412 nm. The absorption of optical emission by BR-containing medium is characterized by the peculiarities due to the change of adsorption sites concentration (molecules in form BR570) as a result of light quantum absorption at wavelength 570 nm by these sites and transition to form M412 with the low-absorption at wavelength 570 nm. As a result absorption in yellow range reduces, medium becomes more transparent-bleached. Intensity of the bleach effect of BR-containing medium depends particularly on the time of relaxation of intermediate M412 molecules to form BR570. Relaxation time is characterized by half-value period of intermediate M412 molecules. Light effect at wavelength 412 nm results to fast coercive transition of molecules to the primary state. The values of photocycle time parameters lie in range from microseconds up to tens of seconds. Optical and dynamical BR characteristics change in wide range by production conditions and matrix composition (medium).

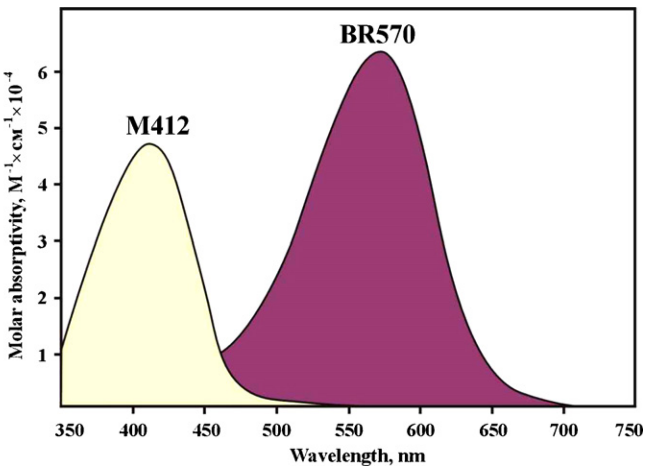

Fig. 2. Absorption spectrum of BR and of the main photocycle intermediate M412.

3. Proposed variant of basic process for classical optical neural network in bacteriorhodopsin medium

It is known a few examples of the experimental neuro-molecular systems based on bacteriorhodopsin—synaptic matrix used of BR photochromic properties [7], synaptic matrix used of BR photoelectric properties [8], artificial photoreceptor [9–11], artificial receptive field [12–14], associative memory [15], three-dimensional memory based on the branched BR photocycle [16]. We propose our approaches to realization of optical neural network based on bacteriorhodopsin medium.

3.1. Non-linear absorption of optical radiation medium bleaching

Absorption of optical radiation in substance is described by known classical equations. Generalized Bouguer–Beer law associates intensities of the incident light and the light transmitted through the substance layer with the layer thickness and molecular concentration of absorption agent:

$$I = I_0\,\mathrm{e}^{-D} = I_0\,\mathrm{e}^{-\alpha d} = I_0\,\mathrm{e}^{-\varepsilon c d} \tag{1}$$

where I is the transmitted light intensity; I_0 the incident light intensity; D the optical density of the substance; α the absorption index of the substance; d the thickness of the substance layer; c the molar concentration of absorbing substance molecules; ε is the extinction coefficient, characteristic feature of absorbing substance molecule.

The values of ε, α and D depend on wavelength of incident light. When the values of coefficients in Eq. (1) are invariable the transmitted light intensity is in direct proportion to incident light intensity.

Non-linear absorption of optical radiation by BR-containing media is connected with the change of absorption centers concentration (molecules in form BR570) as a result of light quanta absorption by these centers at wavelength 570 nm and transform to form M412 with low absorption at wavelength 570 nm. Finally, as mentioned above, absorption in yellow range ($\lambda = 570$ nm) decreases, medium becomes more transparent-bleached [17,18].

3.2. Indirect interaction of optical radiation fluxes in bacteriorhodopsin-containing media [19]

Mediated interaction of optical radiation fluxes appears during the sequential or combined transmission through the same part of BR-containing medium and the most clearly appeared for monochromatic radiation with wavelengths 412 nm and 570 nm.

As a result of interaction between bacteriorhodopsin and the radiation with wavelength 570 nm during the transmission through medium the energy of the light flux is absorbed and in BR-containing medium the photo-induced allocation of the absorption index forms. The light-induced allocation of the absorption index variation corresponds to energy distribution along the surface of the transmitted light wave front (Fig. 3a).

Unmodulated along the front surface light pulse with wavelength 570 nm or 412 nm (as actuating or inhibiting signal) absorbs spatially non-uniformly in compliance with the changed value of the absorption index (Fig. 3b and c).

Thus, energy distribution along the preceding pulse front surface, indirectly, over the BR-containing medium, modulates the energy distribution along the following pulse front surface.

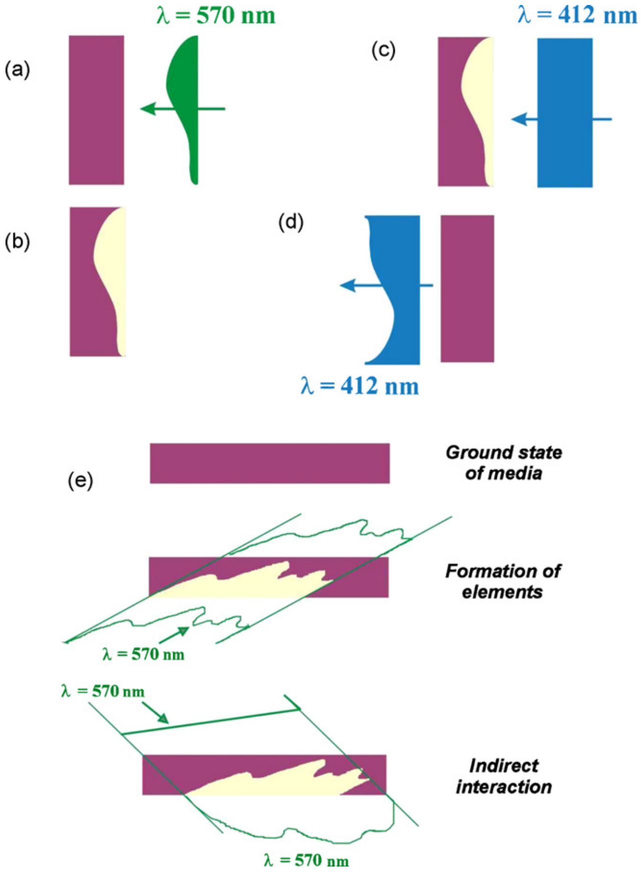

Fig. 3. The indirect interaction of optical radiation fluxes in BR-containing media: (a) green light pulse (modulated along the front surface) acts on a layer of BR-containing medium non-transparent for green light; (b) modulated allocation of molecules in form M412 (transparent for green light) and BR570 (non-transparent for green light); (c) the action of unmodulated blue light pulse; (d) modulated blue pulse as a result of transmission via the modulated medium (BR-containing medium layer recovered the primary state); (e) green light pulse modulation during the transmission via medium.

As a result BR-containing medium is capable to accumulate (to sum up) effects signed “+” and “−”, correspondingly, at wavelength 570 nm and 412 nm. Predicating upon the indirect interaction of optical radiation in BR-containing media the method of formal neuron creation in such media is available to offer. At that all the main functions can be realized by optical technique.

4. One of the possible methods of formal neuron main functions realization in bacteriorhodopsin medium

Realization of the main operations of neuronet algorithms – weighting of input signal vector according to the matrix of weighting coefficients of synaptic bonds, composition of weighted values of input signals, realization of activation (threshold) function by optical method without optoelectronic buffering – permits to simplify the construction and the technological realization of multi-layer optical neuronet, to increase the integration of neuro-like elements in device, to solve the problem of areal density limitation inherent to microelectronic elements and electric connections.

4.1. Formation of the neuro-like element

Neuro-like element is formed under exposure of optical emission with the spectrum, corresponding to the absorption spectrum of BR-molecules initial state, on BR-containing material medium. This element is a part of BR-containing medium with photo-induced absorption index. Threshold properties of such neurons are defined by the concentration ratio of molecules in primary and photo-induced forms and the interaction of neuro-like elements is provided by optical emission.

4.2. Construction

The various solutions of neuronet organization are known for realization of classical neuronet operations based on vector-matrix multiplication by optoelectronic methods [20]. In the general case input image device as a light-emitting diode bar and cylindrical lens, controlled filter as a matrix electrically controlled time–space optical modulator (TSOM), cylindrical lens (for the resulting signal light-front formation) and multi-element photo-detector are used [20].

The example of the similar neuronet realization based on information conversion basic process in BR-containing media (Fig. 4) is considered below.

Construction for optical neuronet formation includes:

1 the source of the plane light front (transparent for normal incident light flux) providing the signal formation and transfer to neurons;
2 photo-detecting layer based on BR-containing material for imaging of the input optical information by photo-induced variation (according to light energy distribution along the surface of input light front) of absorption/transmission in BR-containing medium;

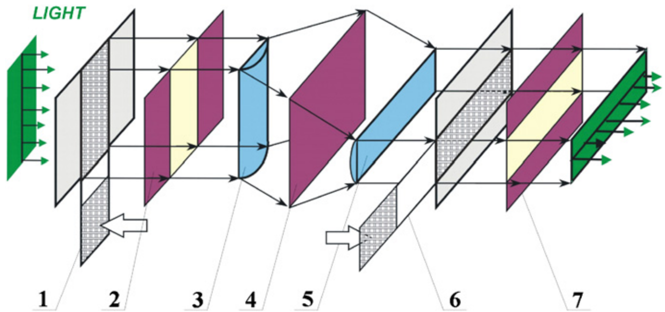

Fig. 4. Neuronet organization based on the basic process: 1 and 6, plane waveguides, including gitters for emission input/output; 2, 4 and 7, BR-containing layers (photo-detecting layer, layer of weighting coefficients and neuron layer); 3 and 5, cylindrical lenses.

3 light flux expanding lens;
4 layer of synaptic bond synthesis (matrix of weighting coefficients) made of BR-containing material;
5 the lens, focusing the light flux on layer 7 and forming like that combination of input optical signals based on neuro-like elements;
6 the source of flat light front (transparent for normal incident light flux) for parallel comparison with thresholds, formation and transfer to other layers of output neuron signals in layer 7 as light front, modulated by intensity along front surface according to the values of absorption/transmission area of BR-containing medium (corresponding to neuro-like elements);
7 the layer of neuro-like optical elements (being obtained on the basis of BR-containing material) composes input optical signals, traversing matrix 4 and realizing the activation function during the light front traverse from the source 6, and forms thereby output signals.

Input optical information in the form of light flux (input vector) effects on photo-detecting layer 2, that results to absorption of light flux energy in BR-containing medium of photo-detecting layer and the distribution of the photo-induced absorption index forms.

Distribution of the photo-induced in BR-containing medium absorption index along the surface and in depth corresponds to power distribution along the surface of the effective light front.

Plane light front from the source 1, modulated by intensity according to the contour of absorption index of photo-detecting layer 2 by lens 3, allocates on the surface of weighting coefficients layer 4.

4.3. Realization of weighting function

Function of input vector weighting comes about during the process of transfer of input light signal – the components of input vector – over the matrix of weighting coefficients 4. By the component of input vector we mean the quantity of light energy (the intensity multiplied by exposure-exposition) affecting on the matrix section.

Weighting coefficient (by that the input vector component is multiplied) is a transmission coefficient of the corresponding section of BR-containing matrix:

$$I_{\mathrm{w}\,ij}t = \omega_{ij} I_{\mathrm{in}\,i} t \qquad (2)$$

where $I_{\mathrm{w}\,ij}$ is the light intensity transmitted over the *ij* matrix section or weighted component of the input vector; $I_{\mathrm{in}\,i}$ the light intensity at the light front section or component of the input vector; *t* the exposition time of the corresponding component of the input vector; ω_{ij} is the weighting coefficient or transmission of the corresponding *ij*-section of the BR-containing matrix.

Transmission of the *ij* matrix section is defined according to Bouguer–Beer law

$$\omega_{ij} = \mathrm{e}^{-\varepsilon d c_{\mathrm{i/f}\,ij}} \qquad (3)$$

where ε is the bacteriorhodopsin absorption factor; *d* the thickness of BR-containing layer; $c_{\mathrm{i/f}\,ij}$ is the concentration of the bacteriorhodopsin molecules in the initial state at the *ij* matrix section.

Values of the weighted components of the input vector are formed by lens 5 in a light flux, which comes to the inputs of the corresponding neuro-like elements of the layer 7.

4.4. Realization of the weighted signals composition function

Composition function of the input signals is realized in BR-containing medium in layer 7 by the converging cylindrical lens 5 as a result of the combined effect at the same area of BR-containing medium of the light energy exposition by the corresponding components of the input vector.

Every component of the input vector contributes to the formation of the molecules ensemble in photo-induced state in proportion to the intensity and exposure time:

$$\Delta C_{j\,\mathrm{p/i}} = k \sum I_{\mathrm{w}\,ij} t \qquad (4)$$

where $\Delta c_{j\,\mathrm{p/i}}$ is the concentration of BR-molecules in photo-induced spectral state; *k* the coefficient of proportionality depending on concentration of BR-molecules in the initial state, interaction cross-section, photoresponse of BR-molecules transition from the initial to the photo-induced form and the effecting light wave length; $I_{\mathrm{w}\,ij}$ the intensity of the weighted *ij*-component of the input vector; *t* is the exposure time of the *i*-component of the input vector.

Thus, $\Delta c_{j\,\mathrm{p}/i}$ contains information about the value of the weighted input interactions sum on the *j*-neuron.

4.5. Realization of the activation function

Magnitude $\sum_i I_{\mathrm{w}\,ij} t$ (the total dose of light energy, effecting on the *j*-area of BR-containing medium) assigns the point at the graph (dependence the transmission value on the sum of weighted input effects) and defines the transmission of the light signal over the *j*-neuro-like element.

The value of the changed transmission $\omega_{\mathrm{n/e}}$ of the *j*-section of BR-containing medium according to the *j*-neuro-like element, assigns the value of the activation function and the output signal of neuro-like element in layer 7 according to Fig. 5. This magnitude depends on molecules number possessing the changed spectral properties on the medium section corresponding to the neuro-like element of the layer 7 according to

$$\omega_{\mathrm{n/e}\quad j} = \mathrm{e}^{-\varepsilon d(c_{\mathrm{i/f}} - \Delta c_{j\quad \mathrm{p/i}})} \tag{5}$$

Graphic chart of dependence of the BR-containing layer relative transmission on the emission energy consists of the area with the initial transmission value (unequal to zero), the area of almost linear transmission change and the saturation area (Fig. 5). In general the curve character corresponds to the activation function proposed for the neuronet realization by Grossberg [20]. The similar compressive function provides automatically the output signal range from 0 to 1 and corresponds to the necessary requirements for realization of re-conversion algorithm during the neuronet learning, for example, according to scheme [20].

Output signal formation of neuro-like element (threshold comparison and realization of the activation function) is carried out by the light front of the specified intensity and duration induced by the source-former 6.

Output signal of neuro-like *j*-element is formed as an energy portion of the active light signal being transferred over the corresponding section of BR-containing medium in layer of neuro-like elements 7 (according to the transmission of the section under consideration) in conformity with the formula:

$$I_{\mathrm{out}\quad \mathrm{n/e}} t_{\mathrm{active}} = W_{\mathrm{n/e}} I_{\mathrm{active}} t_{\mathrm{active}} \tag{6}$$

The minimal value of output signal is fixed by the transmission of non-firing neuron (the value of input signals sum is close to zero) and corresponds to the initial transmission of photochromic medium and the maximal value is close to the value of active front energy and corresponds to the saturation area of the curve (Fig. 5) and to maximal excited state (transmission) of firing neuron. Output signals of neuro-like elements form the continued light front being modulated according to the activation function at every point of BR-containing layer 7. System learning (formation of matrix weighting coefficients) corresponds to formation of adequate values of transmission coefficients of matrix sections based on BR-containing medium that can be achieved by the inverse transformation method.

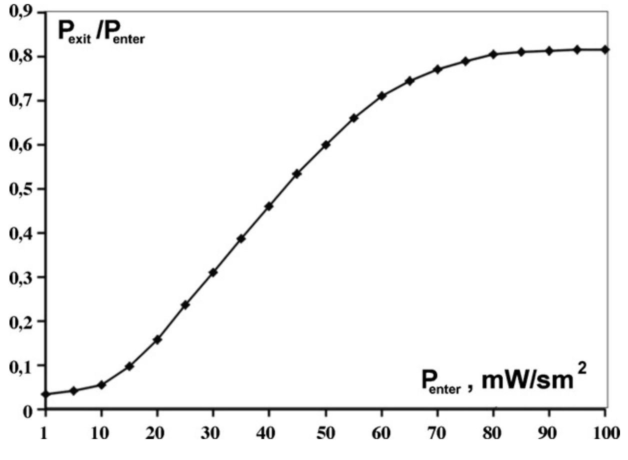

Fig. 5. Transmission ($P_{\mathrm{out}}/P_{\mathrm{in}}$) of BR-containing layer depending on the effective emission energy (P_{in}).

Optical version of the inverse transformation method can be simply and effectively realized (failing optoelectronic transformations) by combined presentation of learning pair: input image in usual direction and the ordered output as light front in the counter direction. Due to the reversibility of light pass both of light fronts will affect on matrix made of BR-containing material and will change the matrix transmission corresponding to intensity distribution.

5. Neuronets based on multilayer optical structures including polymeric BR-containing layers

The approach under consideration of neurocomputer elemental base formation takes into account the cyclicity of processes in living systems appearing, for example, in spontaneous activity of pace making (assigning the rhythm of functioning) neurons. As was conclusively shown in [21] the cyclicity proceeds as a result of self-organization of processes in open non-linear non-equilibrium systems and origination of stable dissipative structures due to that the coordination of trophic processes (providing cell nutrition) is possible in living systems. The number of investigations [22,23] is devoted to the cyclic process self-organization as applied to informational systems.

The similarity of cyclic processes in living cell membranes (including neurons) and the processes under optical emission exposure in isolated purple membranes is obvious, particularly, taking into account that in that case one of halobacteria trophic cycles is reproduced.

If neuron is represented as a structure population (purple membranes) allocated in media and interactions between the neurons are carried out by light fluxes, the “neuro-like medium” under question can be considered as alternative to the net of neuro-like element [24].

In the interpretation being stated the problem of neuronet formation is in simulation of non-equilibrium non-linear systems in bacteriorhodopsin-containing media with allocated parameters by optical emission of two various wave lengths corresponding to absorption maxima of two “long-living” intermediates. It can be solved in the following consequence:

1. Using the spectral sensitivity of bacteriorhodopsin in optical range to initiate the cyclic light-dependent processes by light flux and to control the processes by application of emission with various wavelengths.
2. Stationary and dynamic structurization of bacteriorhodopsin-containing films by modulated light fluxes to create the conditions of formation and development of neuro-like relations in neuro-like medium and to correlate the multitude of cyclic processes.

Data input and processing in that case is “deformation” of the correlated cyclic processes in bacteriorhodopsin-containing medium and the commands of system behaviour control are the transient processes originating in the organized neuro-like medium.

On the other hand, analysing the development tendencies of neuronet technology the most perspective can be emphasized and their combination in the same device can provide essential expansion of neuronet data processing.

1. Optical mechanism of data transmission and processing, which will permit to construct three-dimensional nets functioning simultaneously at high speed. The problem of wide application consists in large dimensions peculiar to optical systems and inevitable efficiency losses due to multiple intermediate optoelectronic conversions and the finite size of optoelectronic elements.The application of continuous photochromic media with high resolution close to molecular level of data representation and processing can become a solution of the problem. Bacteriorhodopsin is the most available and well-investigated (at present) photochromic material with sufficiently high cyclicity ($>10^6$) and suitable for the considered purposes. Using BR enable to carry out data processing in optical mode without intermediate optoelectronic conversions and to increase the areal density of neuro-like elements to value comparable with that for the native neuron systems.
2. Neuro-like structure construction on the principles of self-organization of the processes in open non-linear allocated dissipative systems like biological objects. However in model variant systems require substance transfer proceeding for non-equilibrium conditions maintenance.BR application can solve the problem of substance transfer that complicates the construction and limits the continuous working life of neuro-like media processing. A light-dependent property of that material permits to simulate effectively open non-linear allocated dissipative systems. Optical exposure in the range of 520–650 nm is like the input flux and dissipative properties, in that case, can be provided by the component of trophic cycle that remains invariable in composition of artificial medium (for example, in polymeric matrix) and also by emission exposure in blue light range ($\lambda = 400$–420 nm).
3. Adaptability of elemental base to losses of some elements during the preparation and exploitation compensated by self-organizing and self-modification of neuro-like structures. Application of continuous media based on BR in conjunction with optical methods permits to form neuro-like elements and the bonds between its suit requirements according to the light energy allocation.

Classical methods of light fluxes formation by lens systems result to loss of advantages expected from significant density of neuro-like elements and to dimension restrictions of elements and systems that peculiar to optical computers. Moreover the volume of BR-containing medium is greater the probable process integration is higher. However the emission access to all the molecule groups is more unfavorable as the medium absorption increases.

We proposed multilayer structures including layers based on bacteriorhodopsin for realization of neuronet medium in non-equilibrium non-linear dynamically allocated dissipative systems.

It is supposed that multilayer structures would permit to proceed data processing at level of BR molecules groups by forming neuro-like elements by optical methods in BR-containing media.

Basic processes in BR-medium are defined by light-dependent changes of absorption index allocation profile along the surface of BR-containing polymeric films. In multilayer structures many light fluxes circulate without interaction. This property is usually proved as an advantage of optical methods enabling data processing and transmission in three-dimensional space. At the same time information array in the form of modulated by intensity light fronts indirectly over the reciprocal fluctuation of absorption index local value of BR-containing media sections and local intensity value of light front sections realize concurrent informational interactions in three-dimensional space of multilayer structure.

We would like to consider the possibility of neuro-like element net organization in bacteriorhodopsin medium by optical method using multilayer structures (Fig. 6) including BR-based layers, wave-guide layers and reflecting layers. To allocate light fluxes the system of waveguides, transparent in the optical range, is formed in BR-containing medium. It is possible to input the controlled emission in the form of light front in BR-containing medium, activating at that the groups of neuro-like elements, by producing in waveguides the sections with the disturbed conditions of total internal reflection.

It is expected that multilayer structure will provide not only functioning and interaction of neurons and their ensembles but the generation of new neurons and links (emission output from one layer and penetrating to other layers) between single neurons and neuronets, will permit, according to the information (image) at system output, to connect and to correlate the cyclical processes originating in bacteriorhodopsin medium. At that the process of self-organization of data processing system will proceed.

The realization of adaptability principles of data processing elements and system self-organization will permit to reduce essentially the requirements to the elements and facility in whole, to provide reliable functioning in case of single elements failure.

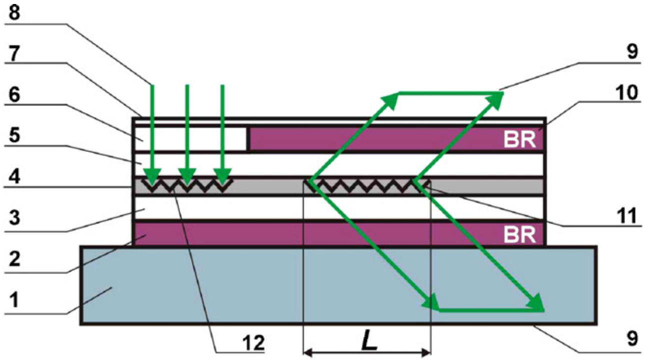

Fig. 6. Fragment of multilayer structure: 1, substrate (glass K-8); 2 and 10, layers containing BR; 3 and 5, boundary layers of flat waveguide; 4, guide layer of flat waveguide; 6, emission input area; 7, adhesive layer; 8, input emission; 9, output emission; 11, output emission gitter, 12, input emission gitter; *L*, the length of the output emission gitter.

Reduction of technological requirements is achieved by that neuro-like elements and links are formed in continuous (uniform, i.e. not divided into constructive matric elements) transparent (without optical dispersion) layer of photochromic material according to the light energy allocation.

According to the traditional criteria it is acceptable to evaluate the number of the neuro-like elements in medium containing bacteriorhodopsin at area 10 mm^2 in quantity not less than 10^6. At area under question not less than 10^{11} bonds are realized per second (circuit time 0.1 msec, coefficient of bond formation 10).

6. Requirements to BR-containing polymeric films in multylayer structures achieved results

To develop photochromic effects during functioning (the induced changes of refraction and absorption indices) high optical density is needed and, consequently, high BR concentration in polymeric films. Optical density of the films under question must lie in the range of 0.8–1.3. In that case the basic properties of BR-containing media are realized at optimal density of light fluxes and can be used in technical purposes.

The induced changes of absorption-transmission in films under consideration amount 10–50% of the primary value under exposure of light fluxes with power density 0.1–100 mW/cm^2 and exposure duration 0.01–10 s.

The homogeneity of allocation of BR concentration resulted from conditions of film functioning in mode of induced absorption fluctuation.

To minimize the influence of diffraction divergence on the process of information conversion thickness of BR-containing films must be 6–14 μm. To reach the specified optical density at considered thickness it is necessary to obtain 40–50% bulk concentration of bacteriorhodopsin in polymeric film.

The film satisfied all requests were been made successfully. Influence of humidity on photochromic properties of the films of bacteriorhodopsin was investigated. Optimal humidity of the films of bacteriorhodopsin was determinated near 70% for best photochromic properties.

For characterization of bacteriorhodopsin films obtained by various methods, differing in their thickness, optical absorption, composition, structure, we introduced a criterion for numerical estimating of sensitivity. By way of that criterion the factor numerically equal to the part of molecules gone from basic state BR570 under settled excitation energy was selected. That factor $k_{570}(t)$ named by us the coefficient of photo-induced transition of bacteriorhodopsin molecules from basic state BR570 was determined from experimental data of changing optical absorption at wavelength 570 nm under acting inducing radiation. Proposed method is based on the kinetic equation evaluating concentration distribution of BR molecules between forms of BR570 and M412. This equation include all parameters characterizing of BR functioning: σ_1, absorption cross-section of BR570 form (on wave-length of acting light); A_1, quantum yield of photoreaction; P, power density of acting light; τ, lifetime of M412 form; ν, acting light frequency. By leading edge slope of time dependence of $k_{570}(t)$ the quantum yield was determined and by falling edge slope of the same dependence was determined the lifetime of M412 form. Sensitivity of bacteriorhodopsin-containing medium may be raised by increasing the quantum yield and the lifetime of M412 form.

As a result of investigations of non-modified films of BR we established that immediately after obtaining the values $k_{570}(t)$ are in interval 0.5–0.7 and than decrease to value 0.2 for 3–5 h and demonstrate this value during few years. In primary formed films BR molecules constitute the structure that is destroyed in time in consequence of the heat oscillations. One of most important task is stabilization of BR films and retaining the highest value of sensitivity during all operation period. Possible decision is the creation of additional linkages (covalent or hydrogenous) between protein molecules by using chemical reagents.

We used few types of bifunctional molecules for stabilization of BR films: aromatic diamines (1,4-diaminobenzene (DAB)), aliphatic dialdehydes (glutaric dialdehyde (GA)) and aminoacids (lysine, glycine and isoleucine).

We studied effect of 1,4-diaminobenzene on photochromic characteristics of BR strain ET1001 films. The samples BR:DAB = 1:3, 1:6 и 1:9 (molecular ratio) were characterized by heightened values of factor $k_{570}(t)$ (0.35–0.5) in comparison with non-modified BR films (0.2–0.25) during 12 days. Stabilization of primary formed BR structure occurs due to the fact that 1,4-diaminobenzene is an aromatic amine and capable to interact with carboxyl groups of glutamic and aspartic acids. In our opinion the high values $k_{570}(t)$ for BR:DAB films are the result of raising lifetime of M412 form owing to the fact that 1,4-diaminobenzene screens extracting proton groups.

We also studied effect of glutaric dialdehyde on photochromic characteristics of BR. For alkaline catalysis of the reaction of bonding between amine groups of BR lysine residues and aldehydic groups of glutaric dialdehyde (the Schiff base) BR: sodium tetraborate in molecular ratio 1:100 was introduced in the initial suspension. The samples BR:GA = 1:5 had more high values of factor $k_{570}(t) = 0.55$ (Fig. 7), whereas BR:GA = 1:10 and 1:30 had $k_{570}(t) = 0.4$–0.5, that is explained by high content of glutaric dialdehyde oxidation products by atmospheric O_2 (glutaric acid) that destroy partially BR molecules. Change dynamics of decay factor $k_{570}(t)$ value was studied during 1 year for BR:GA films (Fig. 7). The samples

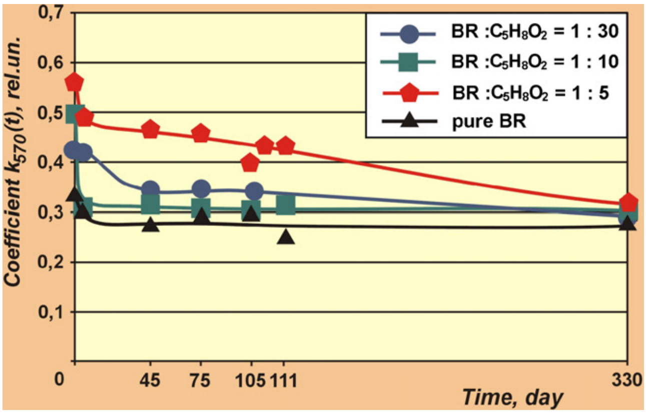

Fig. 7. Changing values $k_{570}(t)$ in the operation process for the films BR:GA = 1:30, 1:10 and 1:5.

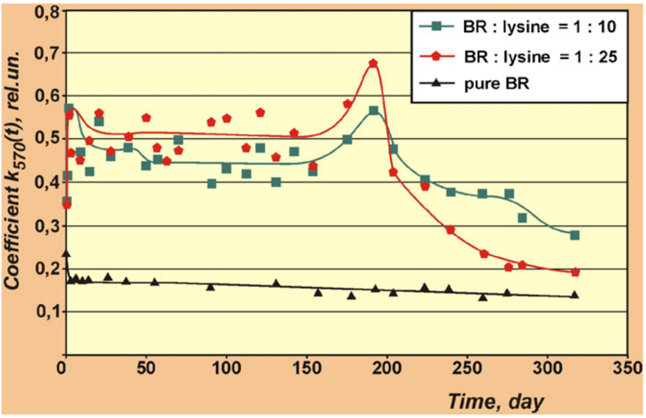

Fig. 8. Changing values $k_{570}(t)$ in the operation process for the films BR:lysine = 1:10 and 1:25.

BR:GA = 1:5 kept high value of factor $k_{570}(t)$ (≥ 0.45) for 120 days. After 330 days for the all samples of BR:GA $k_{570}(t) = 0.3$.

The films of BR:glycine, BR:isoleucine and BR:lysine in molecular ratio from 1:1 to 1:25 had been made. The BR:glycine and BR:isoleucine films were first inhomogeneous optically and characterized by high light scattering, consequently had low values of factor $k_{570}(t)$ within the limits of 0.05–0.1. The BR:lysine films were optically transparent and homogeneous. Dynamics of changing value $k_{570}(10)$ for films with ratio BR:lysine = 1:10 и 1:25 was shown in Fig. 8. The samples with ratio BR:lysine = 1:25 stably kept during not less 80 days more high values $k_{570}(t)$ (0.4–0.55) in comparison with the check sample (0.15–0.2). At that optical density and transparency of films did not change for that time. The samples with ratio BR:lysine = 1:10 demonstrated more high values $k_{570}(t)$ (0.35–0.5) in comparison with the check sample (0.15–0.2) for 275 days.

Obtained experimental results justified hypothesis about stabilization of highest values of BR films sensitivity in the operation process for 275 days due to lysine introducing.

It is important to learn to manage of single intermediate of photocycle for efficient application of bacteriorhodopsin. In our opinion it is possible in nanocomposite materials based on metallic colloids using plasmonic resonance effect. At present time we are experimenting in this area, and we are obtaining first encouraging results.

7. Conclusions

(1) Basic processes and original approaches of neural networks realization based on bacteriorhodopsin in composition of multilayer structures.

(2) Requirements of BR-containing functional films are determined and results of investigation of layers modifications are represented for the purpose of stabilization of extremely high photochromic sensitivity of BR-containing media. The modification of BR-films using bifunctional crosslinking reagents to increase of technological parameters of BR is applied.

References

[1] N.N. Vsevolodov, Biopigment-photographic recorders, in: Photomaterial Based on Bacteriorhodopsin, Science Publ., Moscow, 1988, p. 224 (in Russian).
[2] D. Oesterhelt, C. Brauchle, N. Hampp, Quart. Rev. Biophys. 24 (4) (1991) 425–478.
[3] D. Haronian, A. Lewis, Appl. Opt. 30 (5) (1991) 597–608.
[4] R.R. Birge, J. Phys. Chem. B 103 (1999) 10746–10766.
[5] D. Zeisel, N. Hampp, J. Phys. Chem. 96 (19) (1992) 7787–7792.
[6] F. Siebert, W. Mantele, W. Kreutz, FEBS Lett. 41 (1982) 82–87.
[7] D.P. Shelton, Opt. lett. 22 (1977) 1728–1730.
[8] D. Haronian, A. Lewis, Appl. Opt. 30 (5) (1991) 597–608.
[9] E. Miyasaka, K. Koyama, I. Iton, Sci. Publ. 255 (1992) 342–344.
[10] K. Koyama, N. Yamguchi, T. Miyasaka, Sci. Publ. 265 (1994)762–765.
[11] H. Takei, A. Lewis, Z. Chen, I. Nebenzahl, Appl. Opt. 30 (4) (1991) 500–508.
[12] R.W. Rodieck, J. Stone, J. Neurophysiol. 28 (1965) 833–849.
[13] M. Marr, E. Hildreth, Proc. Res. Soc. London, Ser. B 207 (1980) 187–271.
[14] S.B. Hwang, J.I. Korenbrot, W. Stoeckenius, Biochim. Biophys. Acta 509 (1978) 300–317.
[15] R.B. Gross, K. Can, C. Izgi, R.R. Birdge, SPIE 1662 (1992) 186–195.
[16] Branched photocycle optical memory device, US Patent 5,559,732 (1996).
[17] E.Ya. Korchemskaya, M.S. Soskin, V.B. Taranenko, Quant. Electron. 17 (4) (1990) 448–449 (in Russian).
[18] A.S. Alexeev, S.I. Valyanskiy, V.V. Savranskiy, IOFAN Proc. 38 (1992) 133–150 (in Russian).
[19] E.P. Grebennikov, Mediated interaction of light fluxes in bacteriorhodopsin-containing media, in: Proceedings of the Second Conference on Molecular Physics of Non-Equilibrium Systems, Ivanovo State University, 2000, pp. 235–238 (in Russian).
[20] F. Wasserman, Neuro-computer Technics: Theory and Practice, Mir Publ., Moscow, 1992, p. 240 (Russian edition).
[21] I. Prigozhin, From the Existent to Incipient: Time and Complexity in Physical Science, Science Publ., Moscow, 1985 (in Russian).
[22] A.S. Dmitriev, Chaos and data processing in non-linear dynamic systems, Radio Eng. Electron. 38 (1) (1993) 18–23 (in Russian).
[23] V. Chinarov, Noisy dynamics and biocomputing with non-equilibrium neural networks, in: Fifth International Symposium on Bioelectronic and Molecular Electronic Devices and 6th International Conference on Molecular Electronics and Biocomputing, Sponsored by FED, November 28–30, Okinawa, Japan, 1995, pp. 243–246.
[24] E.P. Grebennikov, Proc. SPIE 3402 (1997) 460–465.